

# Development and validation of analytical methodology for quality evaluation of Tibolone in capsule pharmaceutical form through UV spectrophotometry

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Tibolone is a drug used as a hormonal repository and is very important due to its characteristic of helping to reduce the symptoms triggered by the hormonal alteration during the climacteric period. Although widely used, the Brazilian Pharmacopoeia does not have any information about this hormone. In the literature, only studies with methodologies using high performance liquid chromatography for drug quantification were found. Thus, this study aimed to develop and validate analytical method using UV spectrophotometry for quality control of Tibolone in capsule pharmaceutical form. The present study followed the validation parameters present in RDC 166/2017 and ICH Q2 (R1), with acetonitrile as solvent and 225 nm as wavelength. Linearity showed a correlation coefficient higher than 0.99, precision presented a mean of 97.81% and accuracy reached a recovery percentage average of 97.18%. It was concluded that the method can be used in the quality control of Tibolone capsules, besides having a low cost and a less complex methodology and easier to perform compared to HPLC.

**Keywords:** Tibolone, Quality Control, UV spectrophotometry, development and validation.

## Introduction

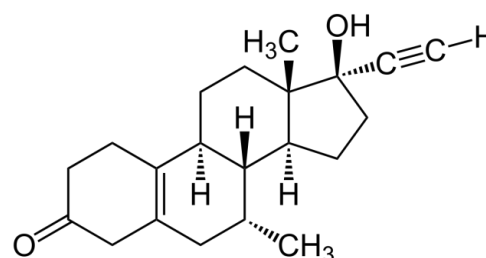
Tibolone is known to be a hormonal repository that has great influence in the prevention of bone loss that usually happens during the climacteric and in the treatment of the symptoms caused by the hormonal alteration. It has estrogenic, androgenic and progestogenic activity. Through the progestogenic activity, the drug provides a protective effect on the endometrium and breasts (1).

The increase in the woman's life expectancy has had a great impact on the health of the female population and in many cases it is essential to adopt measures aimed at a better quality of life during and after the climacteric (2).

Discussions about hormone replacement therapy began around the 1960s, where, for all women who were going through the menopause period, estrogen therapy was prescribed, which itself caused several complications due to the increased endometrial level. In the 1980s, the protective characteristic caused by substances that mimicked the protective effect of progesterone was discovered, and in 1990 the first studies were carried out showing that hormone replacement therapy could aid in the reduction or disappearance of hormonal alteration symptoms. However, it was only in 2002 that the prescription of hormone replacement therapies was initiated, only when the really needed intervention of medication was verified and not only for the reduction of the symptoms. (3).

After oral administration, the absorption of Tibolone is effected rapidly, due to its being widely absorbed, besides having rapid metabolization. Its maximum concentration in the organism is obtained between 1 - 4 hours after the administration. Part of the excretion occurs through the urine, but most of it is eliminated by the feces. (4).

The various changes that have been occurring commonly in the pharmaceutical industry have strengthened the need for innovation and quality assurance, mainly due to the great competitiveness in the sector. In this sense, quality control is no longer seen as a bureaucratic collection, becoming one of the most important processes of obtaining a pharmaceutical form, seeking to guarantee quality, effectiveness, safety and in response to consumer confidence. (5).



**Figure 1.** Chemical structure of Tibolone.

The need to perform a quality control that ensures greater efficiency, effectiveness and safety is related to the uniformity of procedures, processes, equipment, materials, activities or systems, making the development of efficient and fast analytical methodologies a practice of extreme value, providing greater security for users and analysts. (6). Since 2000, the increase in the number of drugs handled in Brazil, mainly due to the need for specific formulation and the lower cost, has led to a greater concern of the Agência Nacional de Vigilância Sanitária (ANVISA) regarding the quality of the products that are being produced and marketed by manipulation pharmacies. The first step was given through the publication of RDC No. 33 on 19 April 2000, which specified instructions on good handling practices in pharmacies. (7).

Currently, RDC No. 67, dated October 8, 2007, is in force, which aims to ensure the safety and quality of formulations produced in handling pharmacies throughout Brazil, in addition to establishing quality control criteria for facilities, equipment, human resources and procedures. (8).

Spectrophotometry is related to the electromagnetic absorption of molecules, and to the concentration and structure of molecules.

UV spectrophotometric methods are widely used in the development and validation of analytical methods for drug analysis. It is important to choose a solvent that does not interfere with the analysis wavelength (9).

Based on this knowledge, the present study aimed to develop and validate a simple, fast and low-cost analytical methodology for the quality control of Tibolone in capsule

pharmaceutical form, based on UV spectrophotometry, since in the literature they are exclusively chromatographic methods.

## Experimental

### Materials

The Tibolone raw material (Lot: TBL / 217008), with declared content of 100%, used as reference standard and 2.5 mg capsules were purchased in a pharmacy of manipulation, located in São Leopoldo, Rio Grande do Sul. The excipients used in the preparation of the capsules were corn starch and silicon dioxide. The solvents used for the development of the method were: acetonitrile (Synth, Vetec, Chemical Group), ethanol (Vetec) and methanol (Synth) PA grade.

### Equipment

The equipment used were UV-vis spectrophotometer model UV-1600 (Pro-tools) and spectrophotometer model UV-2600 (Shimadzu). The glassware used during the experiments were made available by the Universidade do Vale do Rio dos Sinos and the tests carried out in the chemistry laboratories of the University and the Nutrifer Technological Institute.

### Development of the Methodology

Based on the Tibolone monograph available in the British Pharmacopoeia (10), the acetonitrile, ethanol and methanol solvents were initially selected for solubilizing the drug.

To carry out the initial tests, the published study regarding the development and validation of high-performance liquid chromatography (HPLC) methods for Tibolone were used.

In the chromatographic analyzes the wavelength was 204 nm and 205 nm and the solvents employed as mobile phase were acetonitrile and water (65:35 v/v), (60:40 v/v), and acetonitrile, water and methanol (60:30:10 v/v). (11,12,13). A secondary standard was then prepared to test different concentrations to determine the one to present the most satisfactory results. The sample preparation was performed through a pool of 20 capsules. After preliminary tests, a spectrophotometer scan was performed between the wavelengths of 200 and 400 nm using solutions of each of the solvents mentioned above with reference standard, excipients and 200 µg/mL sample to verify the maximum absorption of the drug and possible interferences caused by the excipients. After defining the most suitable solvent to perform the method, it was to determine the most appropriate sample concentration.

### Validation

To perform the validation of the analytical method, the guidelines presented in RDC 166/2017 of ANVISA (14) and the International Conference on Harmonization (15) were followed. The parameters evaluated were: specificity, linearity, precision, accuracy and robustness.

### Specificity

The sample solutions, the reference standard and the simulated mixture of the excipients were scanned at the same concentration in the 200 to 400 nm range in order to evaluate their interference in the quantification of the drug. An amount of powder equivalent to 10 mg of Tibolone was used to prepare the excipient and sample solution, whereas 10 mg of the reference standard was used for the raw material solution.

### Linearity

Linearity was evaluated through the construction of three analytical curves ranging from 60 to 140% of the work concentration. For this purpose, a stock solution of secondary standard (1.0 mg/mL) was prepared from which aliquots were drawn and diluted in 10 mL volumetric flasks with acetonitrile, resulting in concentrations of 120, 160, 200, 240 and 280 µg/mL. It was decided to use 10 mL volumetric flasks in the dilutions to avoid waste and minimize the generation of waste due to the need to use organic solvent. Statistical analysis of the data was performed through ANOVA with a 95% confidence interval.

### Precision

Precision was expressed by repeatability, intermediate precision or reproducibility. Samples were prepared regardless. (16).

To evaluate the repeatability, six independent samples were prepared at a concentration of 200 µg/mL and the absorbance readings were performed in the spectrophotometer. In the intermediate precision the same procedure of preparation of six independent samples of the same concentration was carried out by a different analyst and on different days. The result was expressed by the relative standard deviation value (RSD%).

### Accuracy

Accuracy was evaluated by the method of addition of standard in sample through 9 determinations, contemplating the linear interval of the analytical method. Three levels of addition (25, 50 and 75% of the reference standard) in the sample were performed from the starting point of the curve of 120 µg/mL, in triplicate.

For the calculation of the recovery percentage, the formula available in RDC 166 of 2017 was used (14).

Experimental mean concentration Recovery =

$$\frac{\text{Standard added} - \text{Sample concentration}}{\text{Theoretical concentration added}} \times 100$$

### Robustness

The verification of robustness was achieved by the realization of linearity with variation of the solvent brand and realization of the method in two different laboratories and with different equipments.

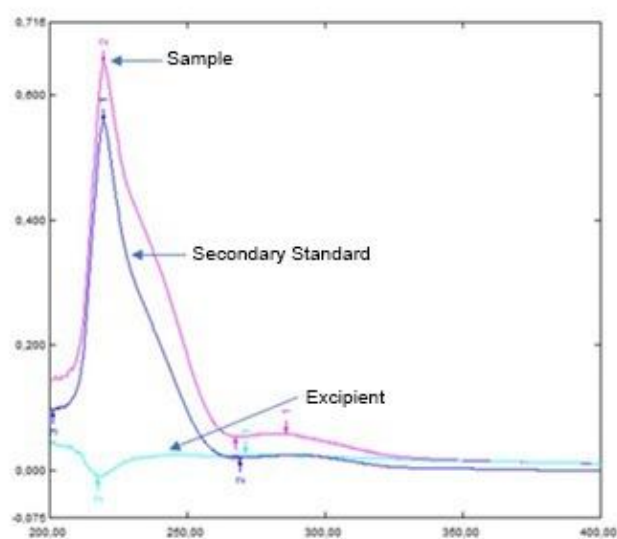
## Results and discussion

Based on the spectra obtained by ultraviolet region scanning, the wavelength of 225 nm was selected for validation of the analytical method, taking into account the maximum absorption of Tibolone and the non-interference of the excipients at this wavelength.

From the tests performed it was observed that it was possible to work by weighing the minimum amount of reference standard (10 mg) using a small volume of organic solvent to prepare the sample.

Through the spectra obtained in the analysis of the solvents, it was verified that ethanol and methanol have absorption peaks in wavelength very similar to the one of the Tibolone, being able to cause interferences in the readings of absorbance of the drug, whereas the acetonitrile does not present peaks that can interfere in the analysis of the drug, basing its choice for development and validation of the method.

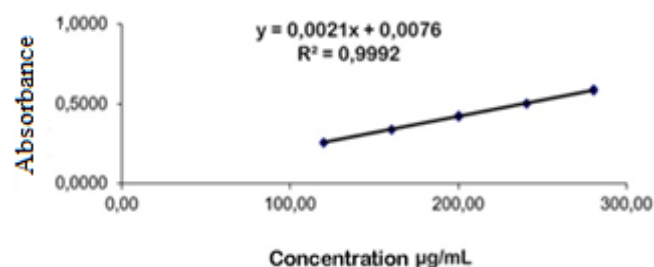
The specificity evaluation was performed by analyzing the spectra of the sample solutions, the reference standard and the simulated mixture of the excipients (200 µg/mL) in the range from 200 to 400 nm, demonstrating that the excipients used in the capsule formulation did not interfere in the quantification of Tibolone in the wavelength of 225 nm, as shown in Figure 2.



**Figure 2:** Absorbance of Tibolone sample, secondary standard and excipients (200 µg/mL) in acetonitrile.

The method was linear with  $R^2$  of 0.9992 and correlation coefficient ( $r$ ) of 0.9996, and the recommended value is a correlation coefficient value higher than 0.99. The equation

of the line was  $y = 0.0021x + 0.0076$  (Figure 3) and in the statistical analysis by ANOVA an F value of 0.37 was found, which was lower than the tabulated value (4.53).



**Figure 3:** Linearity analytical curve.

The repeatability was evaluated by the preparation of six independent samples in the working concentration, while in the intermediate precision another analyst was responsible for the preparation of the six samples. From the results obtained, the values of RSD% were calculated. The repeatability and intermediate precision presented RSD values of 2.32% (day 1), 2.65% (day 2) and 2.92% of RSD intraday (Table 1). The new resolution of ANVISA (14) does not specify a reference value, but the old resolution (17) recommended a RSD value less than 5%.

Accuracy was assessed by adding standard in sample and determined by recovery percentage. According to Table 2, the results obtained were satisfactory, obtaining recovery percentages close to 100%. The accuracy values are between 95 and 105%, assigning an acceptable characteristic for this parameter.

The method remained robust even when the solvent brand variation was performed and also through the results obtained from the tests performed in two different laboratories and with different glassware and equipment, obtaining a difference between the results of less than 1%.

**Table 1:** Precision and intermediate precision results obtained by analysis UV spectrophotometric.

Day	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	RSD %
1	95.55%	97.00%	99.10%	93.03%	94.69%	97.92%	2.32%
2	101.19%	102.23%	97.30%	100.53%	95.22%	99.91%	2.65%
<b>RSD Intraday</b>							<b>2.92%</b>

**RSD:** Relative Standard Deviation.

**Table 2:** Percentage values of Tibolone recovery by adding pattern to sample by UV spectrophotometry method.

	Tibolone ( $\mu\text{g/mL}$ )			Average	Content (%)
25%	34.66	27.44	28.44	30.18	100.6
50%	58.22	56.44	57.77	57.47	95.78
75%	86.55	84.68	85.72	85.65	95.16

**Content:** Recovery percentage.

## Conclusion

Through the analysis of the results, it was possible to verify that the developed method meets the parameters required for validation, obtaining proof of specificity, linearity, precision, accuracy and robustness. Since it is proven that the methodology is satisfactory, it is indicated the use of the same to perform quality control of Tibolone in several areas, but mainly in handling pharmacies and laboratories that do not have sophisticated equipment like a liquid chromatograph and need simpler methodologies as well enabling lower cost and faster analysis, however that are effective and accurately portray the results obtained.

## Conflict of interest

The authors declare no conflicts of interest.

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